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THE TRANSMISSION OF TRYPANOSOMA LEWISI BY RAT FLEAS (CERATOPHYLLUS SP. AND PULEX SP.), WITH SHORT DESCRIPTIONS OF THREE NEW HERPETOMONADS.* †

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After several years of doubt and argument, rigid experiment and accurate observation have brought to definite knowledge the fact that certain invertebrates serve as true intermediary hosts for blood trypanosomes. By the last clause, I do not mean to convey the idea that the trypanosome necessarily passes through a sexual cycle within the invertebrate host. This much has been proved, that it establishes itself in the invertebrate in such a manner as to make possible for an indefinite period its introduction into its vertebrate host by the bite of the intermediate host. In other words, the fact of a cyclical method of transmission has been established. This does not annul the fact that the invertebrate may in addition act as a mere mechanical carrier.

Recent experiments on the tsetse fly and the rat flea have been

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so carefully carried on and with so many positive results, that there can be no doubt that these insects are more than mere mechanical carriers of trypanosomes. In the *Bulletin of the Sleeping Sickness Bureau* (1910), experiments on the tsetse fly are recorded which show beyond doubt that trypanosomes remain infective in the fly for a long time. Minchin and Thomson (1910) have admirably shown by experiment the same to be true of the rat trypanosome in the flea. Those who have read their report must have been impressed with the large majority of positive results obtained under the most rigid control of factors—results which can have no other meaning than that the rat flea acts as an intermediary host for *T. lewisi*. Nuttall (1908) clearly showed that rat fleas and rat lice are capable of transmitting *T. lewisi;* his experiments, however, were not such as would prove whether fleas and lice act as intermediary hosts or merely as mechanical carriers.

Whatever mistakes have been made by confusing trypanosomes with insect flagellates, it is now certain from experiment that the trypanosome does not always degenerate when taken into the stomach of the insect, but on the contrary often remains there for an indefinite period in such condition as to cause infection in vertebrate hosts.

Adding to these experiments the observations of developmental changes which take place in trypanosomes ingested by insect hosts, the evidence in favor of the cyclical method of transmission becomes overwhelmingly convincing. Among the observers who have brought forward strong evidence to this effect are Schaudinn (1904), Prowazek (1905), Minchin (1908), Breinl and Hindle (1909), and Gonder (1910). In addition to their observations, I will show in this paper that *T. lewisi* undergoes a cycle of development in the rat fleas (*Ceratophyllus* sp. and *Pulex* sp.)

Patton has been wisely conservative about accepting the evidence brought forward to show that insects act as true hosts for the transmission of trypanosomes. In various papers, for example, Patton and Strickland (1908) and Patton (1909*a*), he and Strickland have contended that forms described by various investigators as stages in the life history of trypanosomes were really stages of insect flagellates belonging to the genera *Crithidia* and *Herpetomonas*.

Such confusion has undoubtedly occurred on account of ignorance of the life histories and wide distribution of insect flagellates. Their work on these genera has been valuable in throwing doubt on much of the former research, in that it shows the absolute necessity of knowing what natural flagellates are present in the intermediate form which is being investigated as the host or carrier of trypanosomes. Patton's works (1907, 1908, 1909*b*, 1910*a*) and Miss Porter's papers (1909, 1910*a*, 1910*b*) on *Crithidia* and *Herpetomonas* have given such complete knowledge of their life histories that it is now possible to undertake upon sure footing the investigation of the changes which trypanosomes undergo in their intermediate hosts. Patton's criticisms deserve the highest praise for the part they have served in showing the imperative care that must be exercised against drawing unwarranted conclusions.

A complete discussion, in this connection, of the literature giving evidence for and against a cyclical development of trypanosomes within certain invertebrates would make entirely too great a volume. Many excellent works have, therefore, been purposely omitted from this discussion.

In a paper on the rat trypanosome (1907) I described meagerly a few stages of a flagellate from the intestine and rectum of a rat flea, supposing them to be developmental stages of *T. lewisi*. Patton (1909*b*, p. 354) holds that I have described a typical *Herpetomonas*. In a later paper (1909) I accepted Patton's suggestion tentatively. In the present work I will show that Patton was probably correct, a true *Herpetomonas* being present.

The rats of Lincoln, Neb., harbor two fleas (*Ceratophyllus* sp. and *Pulex* sp.).[1] They both contain herpetomonad forms which are so similar that I regard them as one and the same species. To these flagellates I assign the name *Herpetomonas pattoni*, in recognition of Patton's extensive work on insect flagellates. The cycle passed through by the trypanosome is exactly the same in both fleas. Therefore, a separate description of the cycles of the *Her-*

[1] An identification of the species of these fleas was impossible owing to the uncertain knowledge concerning the group. Professor C. F. Baker to whom the fleas were sent informed me that the former is nearest to *C. lucifer* (Roths.), but not that. This form was formerly identified for me as *C. fasciatus*. (See my paper, 1909, p. 1.) The second flea, he informs me, is possibly *P. brasiliensis* (Baker), which may be the same as *P. cheopis* (Roths.) and *P. pallidus*.

petomonas and the trypanosome in the respective fleas will not be necessary.[1]

The flagellates found in the fleas are either pure *Herpetomonas*, pure *T. lewisi*, or a mixture of the two. The following table shows the conditions as they were found to exist.

Ceratophyllus.

	No Flagellates	*Herpetomonas* and *Trypanosoma*	*Herpetomonas*	Developing Trypanosomes	Trypanosomes Not Developing
From infected rats...	5	2	1	7	3
From uninfected rats.	23	2	7	2	..
Pulex.					
From infected rats...	7	3	8	23	..
From uninfected rats.	17	3	25	9	1
TOTAL PERCENTAGES.					
From infected rats...	20.3	8.5	15.2	51	5.1
From uninfected rats.	45	5.6	36	12.3	1.1

It should be observed that a much higher percentage of fleas taken from uninfected rats than of fleas from infected rats have *no* flagellates; that the percentage of fleas harboring *Herpetomonas* alone is likewise higher from uninfected rats than from infected ones; that, on the other hand, the percentage of fleas possessing trypanosomes alone is much lower from uninfected than from infected rats; and, finally, that the percentage of those harboring a mixed infection is about the same in both cases. These results raise certain questions. Why did 20.3 per cent of the fleas taken from infected rats have *no* flagellates at all? Why did 12.3 per cent of the fleas taken from uninfected rats contain trypanosomes? These questions are satisfactorily answered by the fact that uninfected and infected rats associate intimately, especially when a half-dozen of each kind are caught together in the same trap, as was sometimes the case. Fleas may easily crawl from uninfected to infected rats, and vice versa. Thus we would expect to find

[1] The flea, *Pulex* sp., harbors a gregarine. The only forms observed were the late stages of cyst formation. They are found in the lumen of the stomach, as many as 24 in number. About 40 per cent of the fleas were found to be infected. Spores are formed within the cysts in great numbers. Within the spores eight spindle-shaped sporozoites develop. The reason why no other stages were seen lies in the probable fact that the earlier stages develop in fleas younger than any examined. Ross (1909) states that the early stages of the gregarine of the dog flea were found only in very young fleas.

about such conditions as are represented by the given percentages. There are two other factors which will help to explain these percentages. It is possible that some of the rats called uninfected were really infected, but so lightly that the parasites were overlooked; and, secondly, that some might have recently harbored trypanosomes, but had recovered at the time of examination. The fact that Strickland (1909) found, out of 45 fleas taken from 12 rats infected with trypanosomes, only two harboring *T. lewisi*, may be partially explained by the possibility that many of them had recently crawled from uninfected rats on to infected ones, but had not yet bitten the latter. There must, however, have been other reasons, which I will mention later.

DEVELOPMENT OF T. LEWISI IN THE FLEA.

The digestive tract of fleas was carefully dissected out in sodium citrate or normal salt solution and different regions of it examined separately. Permanent preparations were made by teasing the portion containing flagellates in a small drop of sodium citrate or normal salt solution and then inverting the mixture over the mouth of a bottle containing 2 per cent osmic acid for about ten seconds. After drying, the preparations were treated with absolute alcohol and stained with Giemsa's stain or iron hematoxylin. The drying may have destroyed the finer cytological structure, but, comparing with Breinl and Hindle's results from wet methods used on *T. lewisi* in the louse, no structures important in a study on the life cycle have been destroyed.

The trypanosome is taken into the stomach of the flea, where it remains but a short time. Even before the blood has completely lost its structure under the influence of digestion, it is oftentimes impossible to find any trypanosomes. They, however, do not die off. They migrate posteriad into the intestine, where important changes take place. This fact may explain Strickland's failure to find trypanosomes. If he was not careful to tease out the tiny intestine and rectum, he would in most cases have missed the trypanosomes, although they might have been present. In the anterior end of the intestine just posterior to the malpighian tubes are found the most typical, unchanged trypanosomes.

Generally a few are present which conform in every detail to the structure of *T. lewisi* in the blood of the rat (Pl. 1, Figs. 1 and 2). These undergo a series of modifications and divisions in the intestine. The first change to be seen is a diminution in the size which progresses until the parasite becomes quite small, measuring about 10 μ long by 2 μ broad (Pl. 1, Figs. 3–6). As the flagellate becomes smaller the nucleus moves toward the posterior end (Pl. 1, Figs. 4–6). The portion of the body posterior to the blepharoplast becomes absorbed so that the blepharoplast comes to lie clear at the posterior end even against the wall, where it becomes large and rounded and stains densely with Giemsa's stain (Pl. 1, Figs. 5–7). The trypanosome now resembles very closely *T. vespertilionis* in the blood of the bat, described by Gonder (1910). In these forms I have sometimes seen the flagellum ending in a small granule near the blepharoplast. Occasionally these forms degenerate. In such cases a little cytoplasm and a flagellum attached to the blepharoplast are all that remains (Pl. 1, Fig. 8). In most forms which do not degenerate the blepharoplast now moves forward and may be found just posterior to, at the side of, or anterior to, the nucleus. As this takes place the undulating membrane, which has thus far been very prominent, may disappear (Pl. 1, Figs. 9–12). A karyosome is present in the nucleus. Such forms as are represented by Fig. 12 are exactly like adult *Crithidia*. Similar crithidia-like forms have been represented by Gonder as stages in the development of *T. vespertilionis* in the digestive tract of the bat mite, *Leiognathus arcuatus*, and by Minchin (1908) as stages in the development of *T. grayi*. Minchin and Thomson (1910) state that the rat trypanosome changes into a crithidia-like form in the flea.

Division may take place in these forms. The process is similar to division in *Crithidia* (Pl. 1, Figs. 13, 14). Trypanosomes in this stage are often seen attached by their flagellar ends to the intestinal wall, and in this respect simulate *Crithidia* very closely.

Another explanation for Strickland's failure to find trypanosomes in many fleas, even though taken from infected rats, may be found in the fact that the trypanosome changes into a true crithidia-like form. Strickland (1909) states that he discovered *Crithidia* in the

intestine of fleas. His words are: "In 8 fleas (*Ctenophthalmus agyrtes*), from 5 different rats infected with *T. lewisi*, I found *Crithidia ctenophthalmi*." It should be noted that these fleas all came from rats infected with trypanosomes, which fact strongly suggests that his *Crithidia* were really transformed trypanosomes. Patton and Strickland (1908, p. 334) give several figures of *C. ctenophthalmi*, the first four of which are almost identical with my Figs. 7, 9, 10, 11, and 12, which I believe represent true trypanosomes. I have never found any true *Crithidia* present.

The other individuals of the type represented by Figs. 5, 6, and 7, which do not change into the *Crithidia* type, curl upon themselves to form an oval rounded mass. The anterior end with its projecting flagellum is lapped back against the posterior end containing the blepharoplast (Pl. 1, Figs. 15, 16). The contiguous edges fuse and the oblong nucleus which was in the middle of the body now lies opposite the blepharoplast in the larger end of the mass. The flagellum encircles the body. Breinl and Hindle (1909, Figs. 34–36) described a similar form, but were unable to determine whether it developed by the coiling up of a normal trypanosome or in some other manner. I never observed any further change in these forms other than is shown in Fig. 17. It is probable that the flagellum is lost and a rounded cyst results, something like Figs. 18 and 19, the latter being similar to a cyst described by Prowazek (1905, Pl. 3, Fig. 52).

Development of the crithidia-like forms may proceed along two separate lines which come to the same end. Like true *Crithidia*, individuals may agglutinate by the anterior ends, forming rosettes, or they may form cysts directly without agglutination. In the first case the anterior ends gradually become thickened and more rigid, the free flagellum and membrane having been lost (Pl. 1, Figs. 20, 21). In the latter figure the form designated "*a*" is the most completely encysted. It is marked by a strong, apparently stiff, ridge beginning near the posterior margin and passing diagonally across the anterior half of the parasite. The form represented by "*a*" in Fig. 20 resembles very closely herpetomonad stages which are described later (Pl. 3, Figs. 50, 51). The rosettes may become much larger than those figured. They show a marked

resemblance to rosettes from the rat louse, figured by Prowazek (1905, Pl. 3, Figs. 53, 54). The bodies of these forms are not rigid like those of *Crithidia* and *Herpetomonas* rosettes, from which they can be distinguished by their soft flexible appearance. In one flea a rosette of trypanosomes, some with their flagella centrally directed, others with them peripherally located, was found in the stomach.

The direct change of crithidia-like forms into solitary cysts is more common. Unlike the case of *Crithidia* and *Herpetomonas*, solitary cysts are almost exclusively found. In fact, this character is so striking that one can be pretty sure whether he is dealing with the trypanosome, without further observation. The anterior end of a crithidia-like form becomes thickened and compressed toward the posterior end, at the same time often showing a peculiar plastic twist (Pl. 1, Figs. 22–24). Thus the whole anterior end, including membrane, if present, and flagellum, becomes plastic and rolled together. The flagellum often remains in the anterior end as an irregular mass, staining slightly with Giemsa's stain (Pl. 2, Fig. 25). On the edge of the body generally opposite the flagellum and blepharoplast there soon appears a heavy ridge which is somewhat curved and raised so that the cyst becomes more or less triangular in cross-section (Pl. 2, Figs. 26, 27). In many cases another lighter line crossing the heavier one at the anterior end may appear (Pl. 2, Figs. 25 and 28). When seen from the side these cysts somewhat resembling kernels of buckwheat have quite a different appearance. They look like asymmetrical bowls with rounded bottoms, the heavy raised edge being the bottom of the bowl, as can be seen in profile when they roll over (Pl. 2, Figs. 29, 30). These forms measure about 5 μ long by 2 μ wide at the anterior end. The posterior end is always acute. During the formation of these cysts longitudinal division is frequent (Pl. 2, Fig. 31). But, different from the case of *Herpetomonas* and *Crithidia*, when division is complete the two daughter cells separate so that solitary cysts result. Division is a factor in the reduction of size.

The further changes were hard to determine. It is probable, however, that some at least of this type round off to form oval

or round cysts (Pl. 1, Figs. 18, 19). In one flea infected with trypanosomes only, a mass of round forms was found, each containing a single chromatin element (Pl. 2, Fig. 32). The lines of separation between the individual bodies were very faint, and in some places not recognizable.

What becomes of these cysts I am unable to say. Many of them must be passed out with the feces, and may, therefore, be taken up by other fleas or by rats. Cysts ingested by rats might pass through the membrane of the digestive tract and enter the blood stream.

A sexual process in trypanosomes has been described by various authors and discredited by others. No one has yet seen an unquestionable case of conjugation of male and female. Yet figures very suggestive of such a process have been published. Before much change in the size or form of *T. lewisi* has taken place in the rat flea, it is possible that conjugation occurs. In one instance a stage very suggestive of such a process was found (Pl. 2, Fig. 33). The specimen was very well stained, and has been accurately represented. It is possible that the posterior end of one flagellate merely underlies the other. However, there are two significant features which should be noted. The posterior end of the larger female (?), unlike all other adult forms found in the flea, is a large rounded mass twisted half-way round. It contains a large round blepharoplast which is apparently connected with a similar blepharoplast in the smaller male (?) form, by two delicate threads. I believe the anterior end of the smaller individual has no connection with the larger form, but merely underlies its edge. This specimen is very similar to Prowazek's conjugating forms in the case of the rat louse. (See his Pl. 3, Fig. 38, 1905.) No other signs of a sexual development like that described by him could be found.

Of great importance is the question regarding the migration of the trypanosome to the region of the flea's proboscis, whence it may be introduced into fresh rats by the bite of the flea. Minchin and Thomson (1910) have experimentally shown that the trypanosome establishes itself in the flea, where it remains for an indefinite period of time capable of being transmitted to rats by the flea's

bite. They further state, as I have also shown, that the trypanosome establishes itself in the flea by the "multiplication of crithidia-like forms in the rectum." This multiplication begins in the anterior part of the intestine and passes backward to the rectum.

Search was made for trypanosomes passing through the wall of the intestine and rectum. No sign of such a migration was ever observed. Yet Miss Porter (1910*b*) actually saw *Crithidia melophagia* pass through the intestinal wall of the sheep-tick. It is therefore probable that the trypanosome, after it has become a *Crithidia* in structure, passes through the wall and makes its way to the region of the proboscis. I found in a section of the viscera two crithidia-like forms just outside the intestinal wall.

The heads, including the probosces, of several fleas were ground up in salt solution. After smearing and staining, forms as shown in Fig. 34 were sometimes found. These bodies having but one chromatin mass are quite similar to the small oval forms found by Miss Porter in the puparia of the sheep-tick (see her Figs. 79 and 85). They might be forms homologous with the "latent bodies" described by Salvin-Moore, Breinl and Hindle (1908) as a stage in the development of *T. gambiense* and *T. lewisi* in the blood of the rat. If these bodies, which I found in the heads of fleas, are real trypanosomes, doubtless they would, upon being introduced into a fresh rat, like the "latent bodies," develop into typical trypanosomes.

I believe that it is very improbable that the trypanosome makes its way forward from the intestine to the proboscis through the lumen of the digestive tract, having found no evidence of such a path. More extended observations and careful technic will be necessary to reveal the facts of the case.

HERPETOMONAS OF THE RAT FLEAS (CERATOPHYLLUS SP. AND PULEX SP.).

About one-third of all fleas examined were found to be infected with a true *Herpetomonas*. Unlike the transitional forms between the trypanosome and crithidia-like forms, there were no intermediate forms to be found between the trypanosome and the *Herpetomonas*. There can be no doubt, as my figures plainly indicate, that the flea harbors a true *Herpetomonas*.

According to Patton, *Herpetomonas* has three stages in its life history: preflagellate, flagellate, and postflagellate. Working from this point of view, he and Miss Porter have been successful in determining the complete life cycles of several insect flagellates. The preflagellate stage is passed in the anterior portion of the alimentary tract, the flagellates as they develop passing backward and encysting in the rectum. For example, in *H. lygaei*, the preflagellate stage is found in the crop, the flagellate stage farther back, and the postflagellate in the rectum. In the case of the fleas I have made a careful examination of the whole digestive tract back to the malpighian tubes without finding any stage of the herpetomonad cycle, except in two cases where it was probable that forms from the intestine or rectum were accidentally introduced in the preparations during the dissections. These negative results lead me to believe that all three phases of life cycle are confined to the intestine and rectum, i.e., the portion of the digestive tract posterior to the malpighian tubes. In specimens where only a few parasites were found, forms evidently belonging to the preflagellate stage were present.

Preflagellate stage.—The infection probably begins in the anterior part of the intestine with round or oval forms which are ingested cysts only slightly modified. This form enlarges, its cytoplasm becoming more granular (Pl. 2, Fig. 35). At the same time the nucleus enlarges, while its chromatin becomes loose. A flagellum may originate directly (Pl. 2, Figs. 36, 37), or division take place, flagella forming later (Pl. 2, Figs. 38, 39). If division proceeds without a separation of the daughter cells, rosettes are produced (Pl. 2, Fig. 40). The individuals gradually elongate and develop long flagella. Some are more rapid in this process, the result being rosettes made up of young oval forms and typical adults (Pl. 2, Figs. 41, 42). All the younger forms are characterized by a granular cytoplasm and large loose nuclei.

Flagellate stage.—This stage is passed principally in the intestine, although adult flagellates are occasionally seen in the rectum. The adult flagellate (Pl. 2, Figs. 43, 44) is a typical *Herpetomonas* with the blepharoplast near the anterior end and the nucleus about the middle of the body. Like other herpetomonad forms, the

flagellum is inserted in the anterior end, no undulating membrane being present. Its path to the blepharoplast is a clear area as in other herpetomonads. The body may be long and slender (Pl. 2, Fig. 43), more or less spindle-shaped with a pointed posterior end (Pl. 3, Fig. 45), or oval with blunt posterior end (Pl. 3, Fig. 46). The elongated slender forms often show a twisting of the body, as is common in other insect flagellates (Pl. 2, Figs. 41 and 44). In some of the small individuals the posterior end is sometimes notched (Pl. 3, Fig. 47). The nucleus, round or oval, contains a central karyosome. The blepharoplast is rod-shaped, and generally lies transversely in the anterior end of the body. It may, however, pass back near the nucleus as in *Crithidia*. These forms measure, flagellum included, about 20 μ long by 1.5 μ to 3 μ thick. The size, however, varies greatly with the shape of the body. The flagellum may be longer or shorter than the body.

In fresh preparations the adult *Herpetomonas* is easily distinguishable from the adult trypanosome. The former has a rigid body and moves by means of the flagellum alone, while the latter has a very soft, flexible body which exhibits writhing, wormlike movements.

Breinl and Hindle (1909) have described herpetomonas-like forms as stages in the trypanosome cycle within the louse. Some of their figures are more crithidia-like than herpetomonas-like. Others are distinctly herpetomonas-like. They recognized the possibility of a mixed infection of *Herpetomonas* and *Trypanosoma*, but came to no definite conclusion. However, their herpetomonas-like figures, and the fact that fleas harbor a true *Herpetomonas*, strongly suggest that the louse likewise may be infected with a true *Herpetomonas*.

Postflagellate stage.—The cyst formation is very similar to that described for *H. lygaei* by Patton (1908). The adult forms may attach to the walls of the intestine and rectum and undergo encystment or remain free in the tract, where they form large rosettes similar to those described by me in an earlier paper (1907; see Figs. 13 and 14). Encystment is marked by loss of flagellum and a reduction in size due to repeated divisions without much growth (Pl. 3, Figs. 48, 49). The individuals composing rosettes and lining the walls of the tract become rigid and spindle-shaped with

sharp posterior ends. In the earlier stages they also develop thin, clear, winglike sides, while a central area running longitudinally remains thick and dense (Pl. 3, Figs. 50, 51). This area stains much more intensely than the sides. Occasionally, however, a portion of this area where the path of the flagellum lies is marked by a clear area (Pl. 3, Figs. 51, 52). If the forms tend to separate when division takes place so that large rosettes are not formed, the parasites are found either singly, in couplets, triplets, or quadruplets (Pl. 3, Figs. 53–56). In groups of twos, threes, or fours, the central anterior ends become very broad, so that triangular forms result. These forms later round off to produce oval or round cysts (Figs. 53, 54). Other quite common types of encysting forms are represented by additional Figs. 57 and 58. In those represented by the former figure, the path of the flagellum is marked by a clear area, on either side of which is a dark, dense band. In some cases the blepharoplast is evidently extruded (Pl. 3, Figs. 59, 60). It is not at all uncommon to find small round forms with only one chromatin body. In a few instances forms were seen with very heavy walls, staining red with Giemsa's stain (Pl. 3, Fig. 61). The final result of encystment appears normally to be a small round form containing blepharoplast and nucleus, and measuring about 2 μ to 3 μ in diameter (Pl. 3, Fig. 53). The whole intestine and rectum are sometimes packed full of such forms almost exclusively.

No observations were made to determine how this flagellate is transmitted from flea to flea. Miss Porter (1910*b*) has shown by careful observations that *C. melophagia* may be transmitted from one flea to another by the ingestion of cysts from the feces or through the egg from parent to offspring. Since the sheep-tick, a blood-sucking insect, is accustomed to ingesting feces, it is probable that the flea also becomes contaminated in the same manner.

TWO NEW HERPETOMONADS FROM FLIES (CALLIPHORA COLORADENSIS [HOUGH] AND SARCOPHAGA SARRACENIAE [RILEY]).

I wish in this connection to give only short preliminary descriptions of some of the stages of these flagellates. To the flagellate of the first fly I assign the name *Herpetomonas calliphorae*, and to that of the second, *Herpetomonas lineata*.

Herpetomonas calliphorae.—The adults of this flagellate occur in the stomach, or mid-gut, of the fly. They measure, flagellum included, about 30 μ in length by 2 μ in diameter. The body is long and slender, with tapering posterior and anterior ends (Pl. 3, Fig. 62). The nucleus is elongated and lies posterior to the center of the body. It contains many chromatin granules. The cytoplasm is finely granular and contains chromatoid granules. The blepharoplast is generally oval or round, especially preceding division. Longitudinal division is preceded by a thickening of the body. The blepharoplast enlarges, becomes rounded, and divides. The flagella apparently split (Pl. 3, Figs. 63, 64).

Encystment occurs in the ordinary manner. Forms approaching encystment become shortened and thickened (Pl. 3, Fig. 65). Their size is reduced by division (Pl. 3, Fig. 66). The flagellum is lost and small triangular forms with sharp posterior ends are produced (Pl. 4, Fig. 67). Oftentimes these develop a strong darkly staining ridge along one side (Pl. 4, Fig. 68). In this respect they slightly resemble a similar stage in the trypanosome cycle (Pl. 2, Fig. 27). But in the trypanosome the blepharoplast tends to remain nearer the posterior end. In the *Herpetomonas* a clear area marking the path of the flagellum is generally visible. Encystment finally results in the formation of round or oval forms measuring 2 μ to 3 μ in diameter.

The most interesting feature about this *Herpetomonas* is the presence of individuals of the trypanosome type. In them a large round blepharoplast lies against the wall in the rounded posterior end, while the flagellum passes forward along the body and projects a short distance beyond the attenuated anterior end. The body gently tapers with soft curves from the posterior to the anterior end (Pl. 4, Figs. 69–72). The side of the body along which the flagellum runs is often thin and gives the appearance of an undulating membrane (Figs. 70 and 72). These forms measure about 15 μ to 20 μ long. I was unable to find any transitional stages between them and the ordinary herpetomonad type. They resemble very closely one stage of *Rhynchomonas luciliae* figured by Patton (1910; see Fig. 6). But in his form the flagellum never projects beyond the body. Forms similar to his other figures

were not found. Nevertheless, these forms may be *Rhynchomonas* and have no connection with the *Herpetomonas* of the fly.

Herpetomonas lineata.—The forms that I studied were obtained from the digestive tract posterior to the malpighian tubes. The adults are characterized by their extremely long and threadlike tails. The anterior end forms an oval blunt head which tapers posteriorly into a long thread. This thread may be no thicker than a large flagellum, but differs from it in that it is a cytoplasmic projection and is more rigid. It is dragged behind and is capable of slight wavy motions. It may be almost straight and as smooth as a flagellum, or granular and angular (Pl. 4, Fig. 73). In the living condition it is generally perfectly straight or slightly wavy. These forms strongly resemble the attenuated adults of *H. muscae domesticae* and *H. sarcophagae* figured by Prowazek (1905). He gives the length of the former as 30 μ to 50 μ, and says that the latter is very similar. In *H. lineata* these adults reach the astonishing length of 385 μ without flagellum, which is always absent in the longest forms. Many measure over 200 μ. The head generally measures about 2 μ to 3 μ in diameter. The blepharoplast and nucleus are always very plain in the head region. The former is generally rounded, except during division, when it becomes rod-shaped. These extremely long forms are often found in process of division (Pl. 4, Fig. 74). The line of separation begins at the anterior end and evidently passes even out to the tip of the threadlike tail. One extremely large form was found dividing (Pl. 4, Fig. 75). I believe the tail of this one had been broken off. Such cases were common.

These long forms tend strongly toward agglutination. Their flagella become rolled up and contracted in a round mass at the anterior end of the body. This mass stains a deep red with Giemsa's stain (Pl. 4, Figs. 73 and 76). As many as fifty have been seen agglutinated by their anterior ends in perfectly symmetrical rosettes.

From these long forms with short flagella, or none at all, there are gradations all the way down to short spindle-shaped forms with long flagella (Pl. 4, Figs. 77, 78). Intermediate with respect to size is a form which resembles the trypanosome and *Crithidia*.

The blepharoplast carrying the flagellum with it moves clear behind the nucleus, which becomes very long and narrow (Pl. 4, Fig. 79). The body of the flagellate is always very slender and sickle-shaped, the posterior end giving the appearance of a sharp beak. A complete series of gradations from the type illustrated by Fig. 77 to this type is to be found. In Fig. 80 an intermediate stage is represented. This form resembles a *Crithidia*. The flagella of all these forms are always longer than the body. They may all be found agglutinated in rosettes with the long-tailed forms.

Here we have a case in which a true herpetomonad form passes over into the likeness of a trypanosome. The only difference is the lack of an undulating membrane, which, however, is absent in some stages of the trypanosome cycle. Were these forms extremely rare, they would have very little significance. On the contrary, they are as numerous as the other types.

DISCUSSION.

It is important to notice the great similarity between forms represented by Figs. 69 to 72, and the stage in the life cycle of *T. lewisi* represented by Figs. 5 to 7. If the former are herpetomonad forms, then the commonest distinctions, such as position of blepharoplast and presence of membrane, between the two genera break down. Transitions between the two genera are complete. On the other hand, if the former belong to the genus *Rynchomonas*, then the distinctions between this genus and *Trypanosoma* disappear. It might be urged that the forms represented by Figs. 5 to 7 do not belong to the trypanosome cycle. But they must be trypanosomes, because they never occur alone, being always associated with the other stages of the trypanosome cycle, and are connected with them by minute transitions. Someone might suggest that the forms from the fly are trypanosomes mixed in with the herpetomonad forms. This is not probable, because it is hard to conceive how a non-biting fly could become contaminated with trypanosomes.

In this investigation some important connections between the blood trypanosomes and insect flagellates have been brought out.

The evidence is ample that trypanosomes change over into true crithidia-like forms when taken up by insects acting as hosts. Some, it is true, will claim that we are confusing the two genera, and that these crithidia-like forms are natural flagellates of the insect. However, the gradations from a true trypanosome into a true *Crithidia* even in the same rosette are so perfect that it cannot be denied that these crithidia-like forms represent a stage in the trypanosome cycle (Pl. 1, Figs. 20, 21). Moreover, the crithidia-like forms are never found except in association with the typical trypanosome stages like Figs. 2 and 5. Furthermore, the trypanosome, in contrast to its behavior in the blood of the vertebrate, agglutinates in the insect host by its *anterior* end as is the case with *Crithidia*.

Thus we have forms making a perfect transition from the genus *Trypanosoma* to the genus *Crithidia*, thereby breaking down the distinctions between the two genera. The natural *Crithidia* of insects, such as *C. melophagia*, are not known to be connected with any blood trypanosome.[1] But the changing of a blood trypanosome over into a true *Crithidia*, when taken up by an insect host, signifies that the natural *Crithidia* of insects are the more primitive, and that the trypanosomes are merely insect *Crithidia* which have been successfully introduced into the blood stream of vertebrates. Likewise the Leishman-Donovan bodies represent natural herpetomonad forms which have been successfully introduced into vertebrate hosts. It has been shown that these bodies when sucked up by their invertebrate host return in their life cycle to the true herpetomonad type.

The facts given in this paper show that a very close connection exists between the four genera, *Herpetomonas*, *Rynchomonas*, *Crithidia*, and *Trypanosoma*. I do not believe it is wise to reduce them to a single genus, although distinctions between them do break down at various points. There are still great differences between them when their whole life cycles are considered.

[1] Since this article was sent to press, a paper by Woodcock (1910) on Avian Haemoprotozoa has been published. On p. 713 he states that he has discovered a true trypanosome in the blood of a sheep which was harboring sheep-ticks infected with *C. melophagia*. He says: "There can be little or no doubt that the *Crithidia melophagia* is simply a developmental phase of this sheep-trypanosome in its alternate, insectan host." While there is considerable evidence favoring his conclusion, it seems to me that there is still a possibility that *C. melophagia* is a true insect flagellate which has never been successfully introduced into the sheep's blood, and that the trypanosome which he discovered is an entirely distinct form.

It gives me pleasure to acknowledge my indebtedness to Professor Robert H. Wolcott, head of the Department of Zoölogy of the University of Nebraska, through whom the facilities of that laboratory were placed at my disposal for the carrying out of this investigation. I wish further to thank him for the personal interest he has shown and for the suggestions he has offered in the preparation of the manuscript. To Professor Myron H. Swenk of the Department of Entomology and to Professor C. F. Baker of Pomona College, I am indebted for the identification of the fleas. I am also under obligations to Mr. B. W. Coquillet of the United States National Museum, for his service in identifying the flies.

NOTE.

Since this article was sent to the publisher I have received a paper from Swellengrebel and Strickland (1910) on the rat trypanosome. In general the flagellates figured by them from rat fleas are identical with my forms. There are, however, two notable differences; they have not figured any typical adult herpetomonads nor any cysts like those shown in Plate I, Figs. 22–24, and Plate II, Figs. 25–30. A comparison of our papers will reveal the fact that the forms which I have considered to be true herpetomonads are considered by them to be stages in the trypanosome development. On account of this fact their arrangement of the stages of development is quite different from mine. They state that 4 per cent of their control fleas, which were fed upon an uninfected rat, developed flagellates. Thus, their experiment does not preclude the possibility of an infection with a natural herpetomonad. I am still of the opinion that such a form was harbored by the fleas which I investigated. Further experiment is needed to decide this question.

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[1] Not seen by me.

EXPLANATION OF PLATES.

PLATE 1.

All figures are magnified 2,866 diameters.

FIG. 1.—Adult *Trypanosoma lewisi* from the blood of rat.

FIG. 2.—Adult *T. lewisi* from the intestine of the rat flea.

FIGS. 3–7.—Trypanosomes from intestines of fleas showing the successive changes undergone. The blepharoplast and nuclei move together, while the posterior end contracts so that the blepharoplast comes to lie against the posterior wall.

FIG. 8.—Degenerating trypanosome from the intestine of a flea.

FIGS. 9–12.—Crithidia-like trypanosomes from the intestines of fleas. In Fig. 9 the undulating membrane is pronounced; in Fig. 10 it has almost disappeared; while in Figs. 11 and 12 it is entirely absent, the true crithidia form being assumed.

FIG. 13.—Trypanosome from the intestine of the flea, in process of division. The cytoplasm is not yet divided.

FIG. 14.—A later stage in division of the trypanosome in the intestine of the flea. The cytoplasm is more than half divided.

FIG. 15.—Trypanosome from intestine of flea. The body is bent back upon itself.

FIG. 16.—A later stage than is shown in Fig. 15. The contiguous edges have fused so that an oval mass bordered by the flagellum results.

FIG. 17.—The next stage in the rounding-off process of the trypanosome.

FIGS. 18 and 19.—Probably represent the final result of the rounding-off process. In the latter figure the blepharoplast has disappeared.

FIGS. 20 and 21.—Agglutinated trypanosomes from the intestine of the flea. Note the close resemblance to *Crithidia* rosettes.

FIGS. 22–24.—Three successive stages in the formation of solitary cysts from such crithidia-like forms as represented by Fig. 10. In Fig. 24 a diagonal ridge is shown. From intestine and rectum of fleas.

PLATE 2.

All figures, except 40 and 42, are magnified 2,866 times.

FIGS. 25–28.—Other types of solitary cysts formed from trypanosomes. The broad ends are anterior. In Figs. 25 and 28 two ridges crossing each other at the anterior end are shown. In Figs. 25–27 note the blepharoplast pressing the wall out to form a spine.

FIGS. 29 and 30.—Side views of solitary cysts similar to those represented by Figs. 25–28. The upper ends are anterior.

FIG. 31.—Division of trypanosome preceding the formation of cysts. From the intestine of flea.

FIG. 32.—Probably a mass of trypanosome cysts. The lines of separation between the individuals are barely visible.

FIG. 33.—Possibly male and female trypanosomes in process of conjugation. From intestine of flea.

FIG. 34.—A form found in crushed head of flea infected with trypanosomes.

FIG. 35.—Young stage of *Herpetomonas pattoni*, the flagellum not having yet formed. From intestine of flea.

FIGS. 36 and 37.—Little later stages of *H. pattoni* in which flagella have developed. From intestine of flea.

FIGS. 38 and 39.—Showing division of young *H. pattoni* and the formation of flagella. From intestine of flea.

FIG. 40.—Rosette of young herpetomonads formed by the division of such a form as Fig. 38. ×2,400.

FIGS. 41 and 42.—Rosettes of herpetomonads, the flagellated forms of which represent later stages than are shown in FIG. 40. Fig. 42 is magnified 2,400 diameters.

FIGS. 43 and 44.—Typical adult *H. pattoni*. In the latter figure note the twist in the posterior end of the body. From intestine of flea.

PLATE 3.

All figures except 62, 63, and 64 are magnified 2,866 diameters.

FIGS. 45 and 46.—Other adult *H. pattoni* from intestine of flea. In Fig. 45 the anterior end is blunt, while the posterior end is pointed. In Fig. 46 the opposite is true.

FIG. 47.—Adult herpetomonad with the posterior end notched.

FIGS. 48 and 49.—Division forms preceding encystment. In Fig. 48 the flagella have disappeared.

FIGS. 50–52.—Herpetomonad forms preparing for encystment. Note the thin winglike sides and a dense longitudinal band. In Fig. 51 a free flagellum still persists on one of the individuals. In Fig. 52 the path of the flagellum in one individual is marked by a light area. From intestine of flea.

FIGS. 53–56.—Encysted herpetomonad forms, which are respectively single, double, triple, and quadruple. In Fig. 55 note the broad anterior ends centrally located. From rectum of fleas.

FIGS. 57 and 58.—Common types of encysted *H. pattoni* from rectum and intestine of rat flea. In the former figure note the broad anterior and sharp posterior end. The path of the flagellum is marked by a clear area bounded on either side by a dense band.

FIG. 59.—Herpetomonad cyst from which the blepharoplast is being extruded. From rectum of flea.

FIG. 60.—Herpetomonad cyst from which the blepharoplast has probably been extruded. From rectum of flea.

FIG. 61.—Herpetomonad cyst with thick wall. Nucleus and blepharoplast both present. From rectum of flea.

FIG. 62.—Adult *Herpetomonas calliphorae* from the stomach of the fly, *Calliphora coloradensis*. ×2,000.

FIG. 63.—Adult *H. calliphorae* with flagellum splitting at its base. ×2,000.

FIG. 64.—Adult *H. calliphorae* with dividing blepharoplast and flagellum splitting in the middle. ×2,000.

FIG. 65.—*H. calliphorae* with broad posterior end.

FIG. 66.—*H. calliphorae* in which the blepharoplast has moved clear to the posterior end and the size of the body has been reduced.

PLATE 4.

FIGS. 67 and 68.—Cysts of *H. calliphorae* from the intestine of the fly. Note the broad anterior ends. In the former figure the blepharoplast is posterior to the nucleus. In the latter figure there is a longitudinal curved ridge. ×2,866.

FIGS. 69–72.—Trypanosome-like forms from the digestive tract of the same fly as contained the above *Herpetomonas*. Note that the blepharoplast lies against the posterior wall and that an undulating membrane is somewhat distinct. The flagellum projects beyond the anterior end of the body. ×2,866.

FIG. 73.—Adult forms of *H. lineata* agglutinated by their blunt anterior ends. Note the extremely long threadlike posterior ends. The upper individual contains many granules in the threadlike projection. From intestine of fly, *Sarcophaga sarraceniae*. ×700.

FIG. 74.—Division of adult *H. lineata* from intestine of fly. ×1,200.

FIG. 75.—Very large form of *H. lineata* just beginning division. The posterior end is probably broken off. ×2,000.

FIG. 76.—Adult *H. lineata*. Note the long pointed posterior end, and the round body at the anterior end, which is the contracted flagellum. ×1,200.

FIG. 77.—Typical adult form of *H. lineata* with free flagellum. ×1,200.

FIG. 78.—Ovate form of *H. lineata* possessing a very long free flagellum. ×2,000.

FIG. 79.—Sickle-shaped form of *H. lineata*. Note the long slender body with sharp posterior end and a long flagellum which passes along the body back to the blepharoplast in the posterior end. ×2,000.

FIG. 80.—Transitional form between the ovate and the sickle-shaped forms represented by Figs. 78 and 79. ×2,000.

PLATE I.

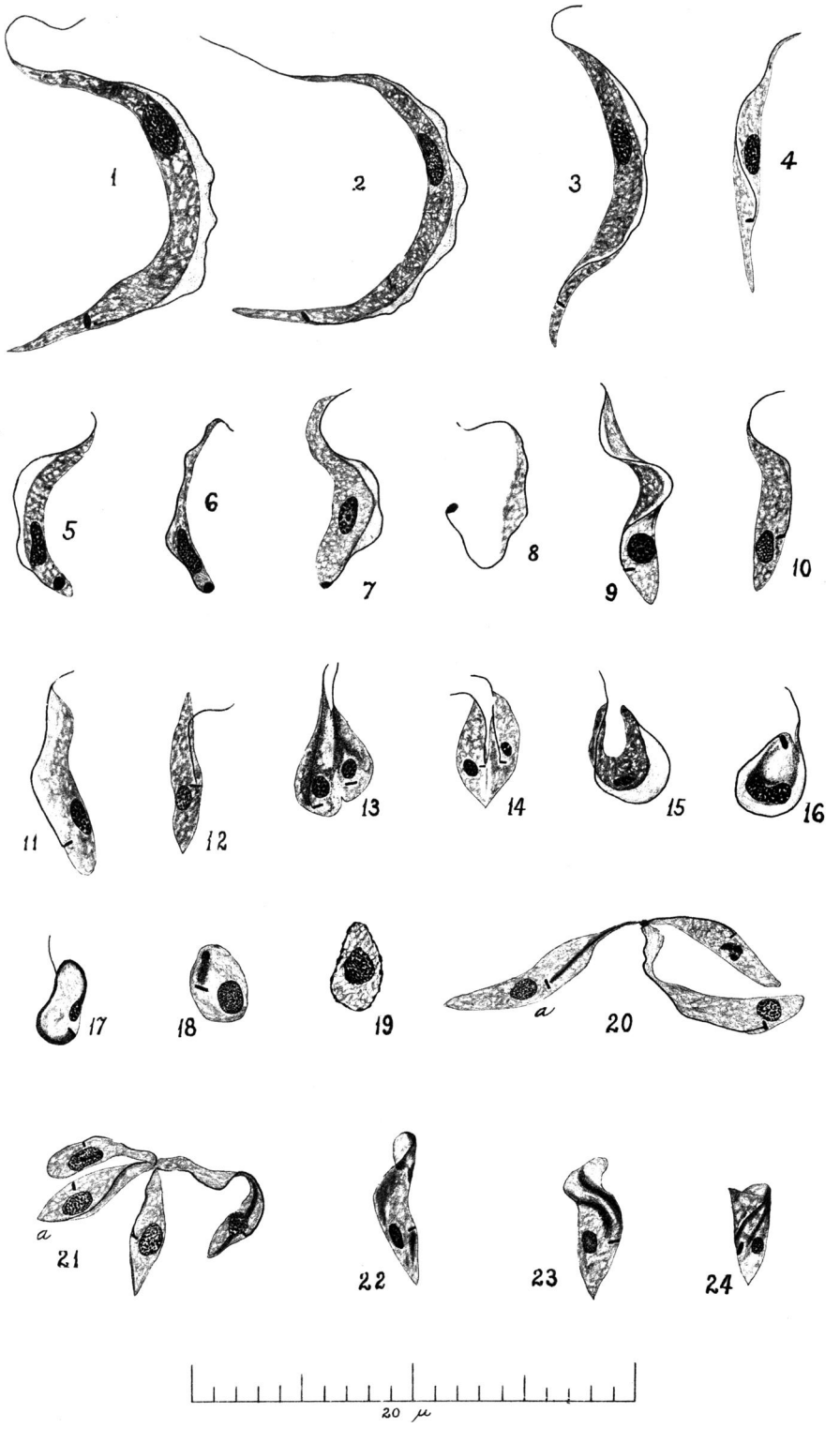

PLATE II.

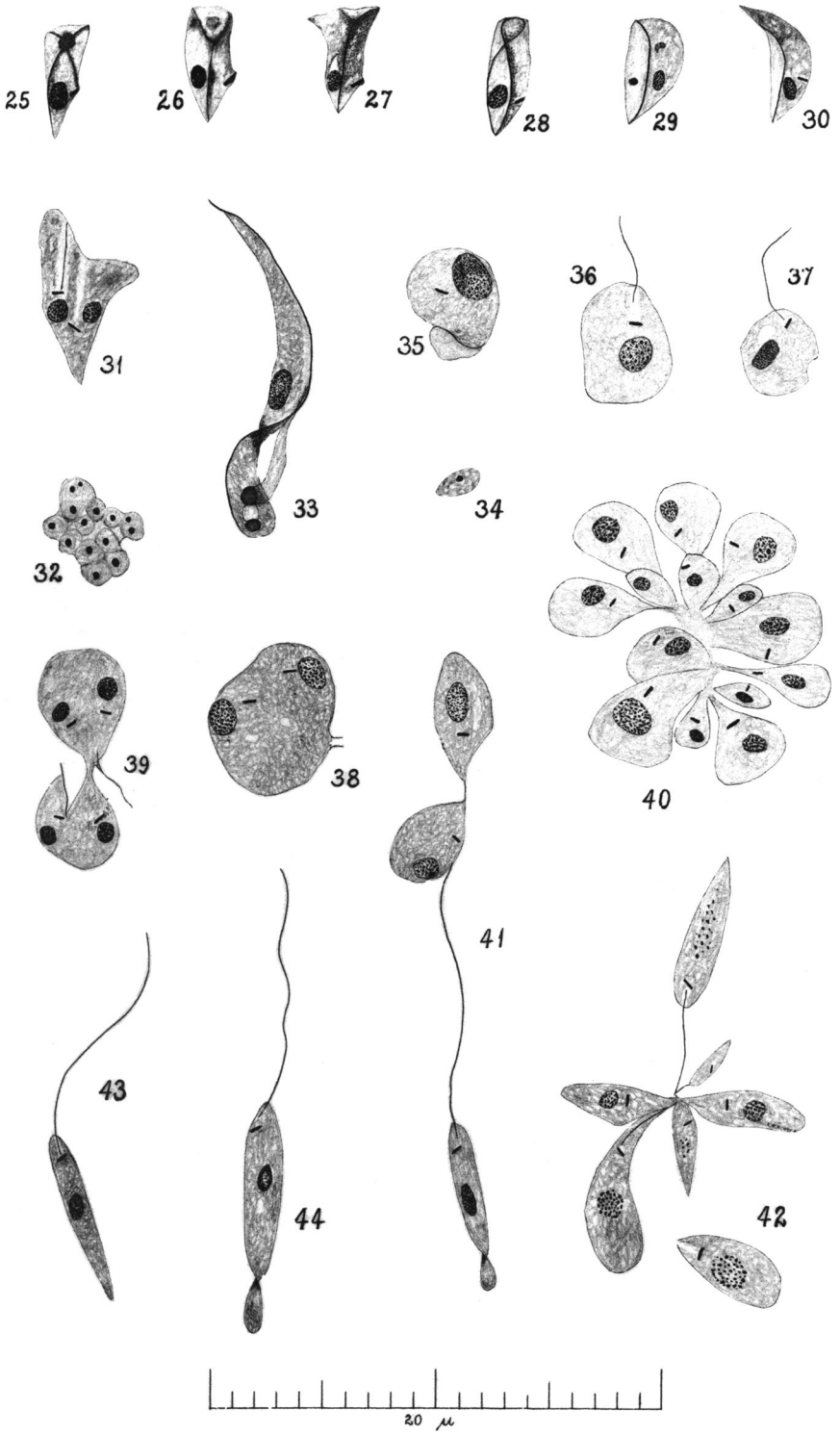

PLATE III.

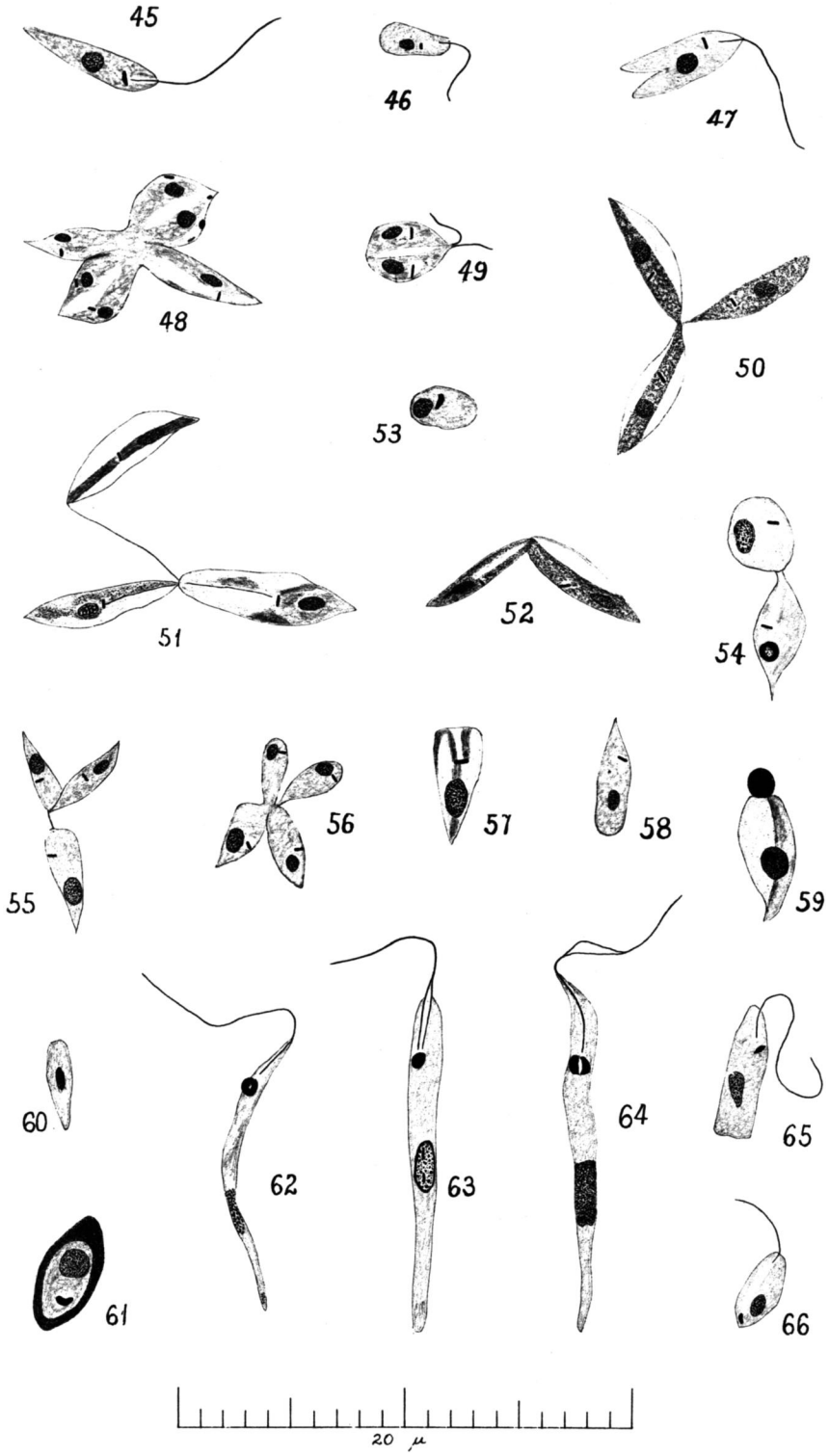

PLATE IV.